Seed germination of *Hypoxis hemerocallidea*

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Accepted 20 January 1988

Seed dormancy of *Hypoxis hemerocallidea* is the result of both a coat-imposed and embryo dormancy. The former is apparently largely affected by mechanical restriction by the darkly pigmented covering structures. While white light improved seed germination of coatless seed it is clear that other restricting factors also contribute to seed dormancy. Chemical treatments enhanced germination and indications are that gibberellins and cytokinins may play a role in the release of embryo dormancy.

Saadrus by *Hypoxis hemerocallidea* is die gevolg van beide 'n saadhuid- en embrio-effek. Eersgenoemde word waarskynlik hoofsaaklik deur meganiese weerstand deur die swart saadhuid teweeggebring. Terwyl wit lig ontkieming van huidlose saad bevorder het, is dit nogtans duidelik dat ander beperkende faktore ook tot saadrus bydra. Sekere chemiese behandelings het ontkieming bevorder en daar is aanduidings dat gibberelliene en sitokiniene 'n rol in die opheffing van die embriorus mag speel.

Keywords: Germination, *Hypoxis hemerocallidea*, seed coat
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Introduction

Extracts from the corms of *Hypoxis hemerocallidea* Fischer and Meyer, previously *H. rooperi* T. Moore (Hilliard & Burtt 1986), have been reported to be effective in the treatment of diseases of the urinary system (Warren 1972). The potential importance in the pharmaceutical industry necessitates an efficient means of propagating these plants. The use of tissue culture has been examined (Page & van Staden 1984). Plants of *H. hemerocallidea* produce many seeds which are apparently dormant. Heideman (1979) reported that seedling emergence of this species could not be observed until a year after planting and that treatments with gibberellic acid (GA_3) were ineffective in promoting germination. Wilsenach (1967) found that treatments of chipping and abrading the seed coat, soaking in sulphuric acid and alternate periods of light and dark failed to promote germination. An understanding of the causes of the low germinability of these seeds may facilitate procedures for the improvement of germination on a commercial scale.

In this study seeds of *H. hemerocallidea* were subjected to a range of treatments in order to stimulate germination and to determine the causes of low germinability.

Material and Methods

Plant material

Mature seeds of *H. hemerocallidea* were gathered from the environs of Pietermaritzburg. Seeds were stored in brown paper bags under laboratory conditions. When required the seeds were sorted into those that were denser than water and those that floated on water, by shaking them in a vial of water for 30 s. Only seeds denser than water were used.

The seeds used in studies related to the seed coat were collected in January 1984 and stored for 7 months. Seeds used in all other studies were collected during November and December 1984 and were stored for periods from 2 to 8 months.

Germination procedures

Seeds were incubated in petri dishes on moist filter paper. The filter paper was moistened with 3 cm^3 distilled water or the appropriate test solution. Where darkness was required, metal foil was wrapped around the petri dishes and all subsequent manipulations, including germination counts, were conducted under a safe green light. At intervals, distilled water was added to maintain moist conditions. For light conditions, white light provided by cool white fluorescent tubes (138 $\mu E\ m^{-2}\ s^{-1}$) were used.

The germination of intact seeds was defined as the rupture of the testa by the elongation of the hypocotyl. The germination of coatless seeds was defined as the noticeable elongation of the hypocotyl. Germination counts were made with the use of a dissecting microscope. Seeds were removed once they were observed to have germinated.

Intact seeds were incubated for 90 days. Coatless seeds were incubated for only 28 days, due to heavy fungal infection of material incubated beyond this time.

For all germination tests 10 replicates of 10 seeds each were used per treatment. Significant differences were calculated at the 95% confidence level. A significant difference among a group of treatments was determined by an analysis of variance. Where appropriate, least significant differences were calculated or comparisons made between pairs of treatments by a *t*-test (Rayner 1967).

Imbibition studies

Water uptake by the seeds was examined at 20, 25 and 30°C in the dark. For each temperature 100 seeds were used, with each seed being massed individually to 0,1 mg on a daily basis. Seeds were incubated on moist filter paper and were blotted dry before being weighed. For each weighing they were removed from the incubation medium for about 1 min.

Control germination levels

For the studies concerning the involvement of the seed coat in dormancy, intact seeds incubated at 25°C in the dark were used.

For the remaining germination studies, a number of control tests were made to ascertain that the control level of germination did not vary with the period of storage. Tests were conducted on 2 February 1985 and on fortnightly intervals until 6 July 1985, a total of 12 dates. On each date, both intact and coatless seeds were tested for germination under conditions of darkness at three temperatures: 20, 25 and 30°C.

Experiments related to coat effects

For these experiments germination tests were conducted at 25°C in the dark. Physical scarification by abrasion between pieces of carborundum paper was performed at two levels. Firstly, the seed coat was much roughened and secondly, the coat was damaged until it broke open and could be removed completely.

Chemical scarification was achieved by soaking seeds in 8,8 M HCl or in 1 M NaOH. Seeds were soaked in HCl for 1, 5, 10, 15, 30, 60, and 90 min respectively. Immediately after scarification the seeds were thoroughly rinsed in distilled water and incubated for germination.

Application of exogenous substances

The compounds and range of concentrations used are shown in Table 1. Treatments were conducted in the dark with both intact and coatless seeds at three temperatures: 20, 25, and 30°C. Treatments marked in Table 1 with an asterisk were conducted at 30°C under the light conditions previously described. Treatments in the light were conducted with coatless seeds only, except for GA_3 and BA treatments, which were conducted with both intact and coatless seeds.

Table 1 Compounds applied to intact seeds of *H. hemerocallidea* incubated in the dark at 20, 25 and 30°C. Compounds marked with an asterisk (*) were also applied to seeds incubated under white light at 30°C

Compounds used	Concentrations applied
Gibberellic acid (GA_3)*	3×10^{-3}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} mol dm^{-3}
6-benzylaminopurine (BA)*	3×10^{-3}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} mol dm^{-3}
Kinetin	10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} mol dm^{-3}
2-chloroethylphosphonic acid* (ethrel)	1 000; 500; 100; 10; 1,0; 0,1; mg dm^{-3}
Potassium nitrate*, potassium nitrite, sodium azide, 2-mercaptoethanol	10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} mol dm^{-3}
Hydroxylamine hydrochloride	10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} mol dm^{-3}
Ammonium chloride*, thiourea*	10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} mol dm^{-3}
Potassium cyanide*	10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} mol dm^{-3}
Hydrogen peroxide	10, 5, 1, 5×10^{-1}, 10^{-1}, 10^{-2} mol dm^{-3}
Ethanol*	5, 1, 5×10^{-1}, 10^{-1}, 10^{-2}, 10^{-3} mol dm^{-3}

In the case of KNO_3 and H_2O_2 treatments, the test solutions formed the medium of moist incubation for 18 h only, seeds then being rinsed in distilled water and transferred to standard germination test conditions with distilled water. With all other substances, the test solution formed the medium of incubation throughout the duration of the germination test.

A combination of GA_3 at 10^{-3} mol dm^{-3} and BA at 10^{-4} mol dm^{-3} was examined with coatless seeds incubated at 30°C in the dark and under white light.

Results

The shiny waxy appearance of *H. hemerocallidea* seeds did not prevent imbibition. Moist incubation in the dark, a situation under which very few seed germinated, resulted in no significant difference in mass increase at the three incubation temperatures tested (Figure 1). The pattern of water uptake between the different treatments was similar, the bulk of the water being imbibed over the first 10 days of incubation.

The intact seeds, irrespective of storage time or incubation temperature, did not germinate after 90 days incubation in the dark or the light. Complete removal of the seed coat by mechanical means did, however, effect some germination. This germination, although still low, was significantly improved by higher incubation temperatures (Figure 2). Mere roughening of the seed coat did not bring about germination. Partial scarification with acid and alkali also had no effect on germination (results not shown).

Complete removal of the seed coat and subsequent incubation under white light at 20°C for 28 days resulted in 36% of the seeds germinating compared to the 6% which germinated in the dark (Figure 3).

As was the case for all the other treatments, no germination was observed when intact seeds were treated with the list of chemicals as outlined in Table 1. This was the case under both light and dark conditions. Coatless seeds did, however, respond to the various treatments in the dark. However, this response was in no case significantly

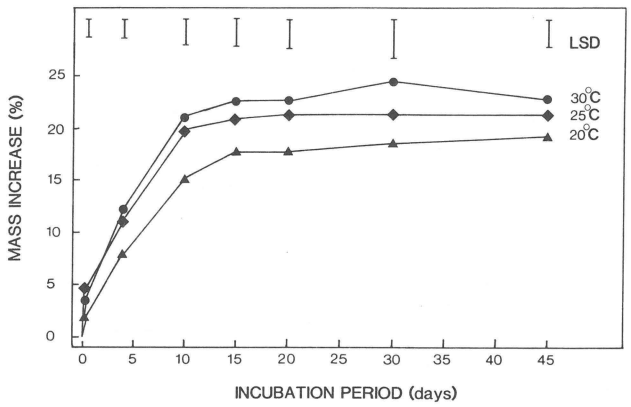

Figure 1 Water uptake of intact *H. hemerocallidea* seeds incubated in the dark. Values not connected by the same vertical line differed significantly ($P = 0,05$).

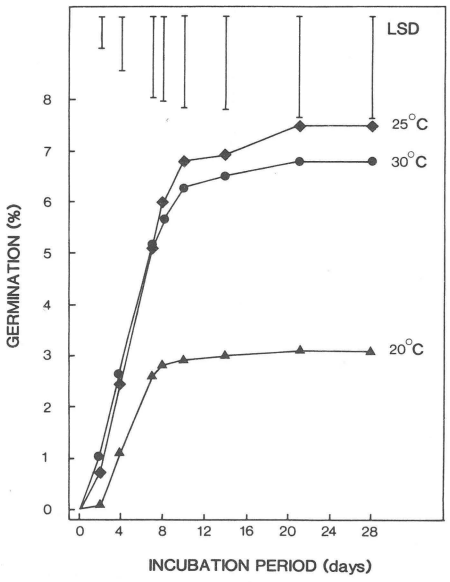

Figure 2 The effect of incubation temperature on the germination of coatless seeds of *H. hemerocallidea*. Values not connected by the same vertical line differed significantly ($P = 0,05$).

enhanced when incubation was in the light. For this reason only the dark-imbibed treatments are considered.

No significant responses were obtained with intact seeds with the application of kinetin, KNO_3, KNO_2, KCN, Na-azide, hydroxylamine hydrochloride, H_2O_2, 2-mercaptoethanol, ethanol or ethrel at the concentrations indicated in Table 1 at any of the incubation temperatures investigated (20, 25, 30°C) (results not shown).

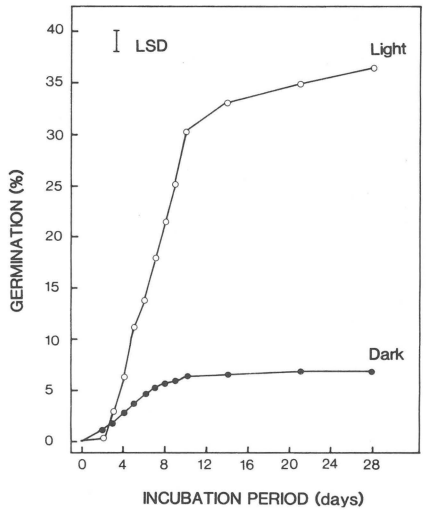

Figure 3 The effect of white light on the germination of coatless seeds of *H. hemerocallidea*. Values not connected by the same vertical line differed significantly ($P = 0,05$).

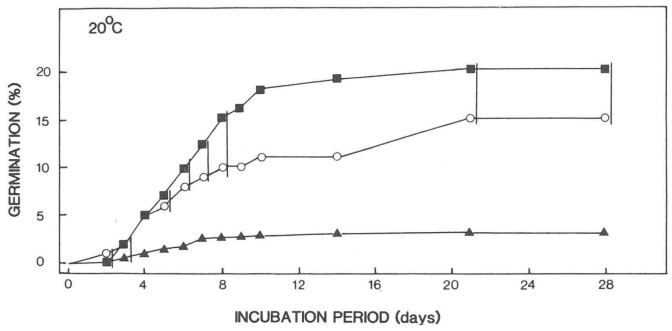

Figure 4 Optimum germination of coatless seeds of *H. hemerocallidea* incubated at 20°C in the dark. Germination values not connected by the same vertical line differed significantly ($P = 0,05$). ▲ = control; ○ = GA_3 at 10^{-4} mol dm^{-3}; ■ = thiourea at 10^{-2} mol dm^{-3}.

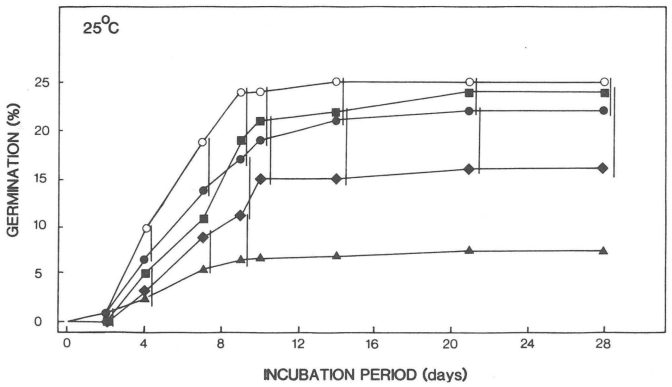

Figure 5 Optimum germination of coatless seeds of *H. hemerocallidea* incubated at 25°C in the dark. Germination values not connected by the same vertical line differed significantly ($P = 0,05$). ▲ = control; ○ = GA_3 at 10^{-3} mol dm^{-3}; ■ = thiourea at 10^{-2} mol dm^{-3}; ● = BA at 10^{-4} mol dm^{-3}; ◆ = NH_4Cl at 10^{-4} mol dm^{-3}.

At the lowest incubation temperature only GA_3 and thiourea improved germination significantly. Significant increases in germination were recorded for GA_3 at 3×10^{-2} to 10^{-5} mol dm^{-3}. The highest germination value was obtained with GA_3 at 10^{-4} mol dm^{-3} and thiourea at 10^{-2} mol dm^{-3} (Figure 4), the response with thiourea being greater than with GA_3 at this temperature.

Germination at 25°C was significantly stimulated by GA_3 at 10^{-3} mol dm^{-3} (Figure 5), the final germination recorded being higher than that recorded at 20°C (Figure 4). This was also true for the thiourea treatments. While no response was observed with BA or NH_4Cl at 20°C these compounds gave 22% (BA at 10^{-4} mol dm^{-3}) and 16% (NH_4Cl at 10^{-4} mol dm^{-3}) germination at 25°C. When incubated at 30°C, similar results were recorded for all the treatments applied. The best response, although not significant, was obtained when GA_3 (10^{-3} mol dm^{-3}) and BA (10^{-4} mol dm^{-3}) were combined (Figure 6).

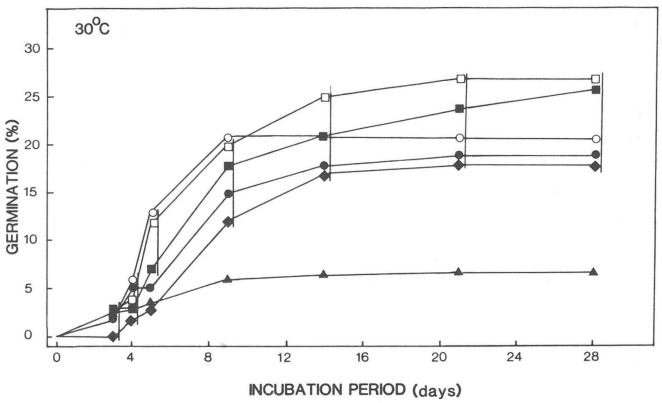

Figure 6 Optimum germination for coatless seeds of *H. hemerocallidea* incubated at 30°C in the dark. Germination values not connected by the same vertical line differed significantly ($P = 0,05$). ▲ = control; ○ = GA_3 at 10^{-3} mol dm^{-3}; ■ = thiourea at 10^{-4} mol dm^{-3}; ● = BA at 10^{-4} mol dm^{-3}; ◆ = NH_4Cl at 10^{-4} mol dm^{-3}; □ = combined GA_3 at 10^{-3} mol dm^{-3} and BA at 10^{-4} mol dm^{-3}.

Discussion

The present study indicates that both coat-imposed and embryo dormancy contribute to the poor germination of *H. hemerocallidea* seeds. Although the intact seeds imbibed water they did not germinate in either the light or the dark. As coat removal resulted in some germination it would seem as if the hard seed coat may mechanically restrict germination. The stimulation of germination after exposing coatless seeds to light and to different chemical treatments clearly points to some kind of embryo dormancy. This embryo dormancy could not be overcome totally by exposure to only white light. The seed coat of *H. hemerocallidea* is darkly pigmented and this may contribute to the inability of light to reach the embryo. In this study the exact role of light in seed germination was not examined. It is, however, significant that none of the chemical treatments applied could improve the response of the seeds to white light. This inability of chemical treatments which significantly enhanced germination in the dark, to further enhance germination under white light, suggests that those seeds responsive to light were also responsive to those chemicals which significantly stimulated germination.

The ability of potassium cyanide, sodium azide, potassium nitrate, potassium nitrite, hydroxylamine hydrochloride, hydrogen peroxide and ethanol to enhance seed

germination in many species is considered to result from their enhancement of the activity of the pentose phosphate pathway (Roberts & Smith 1977). The failure in every case of these compounds to stimulate germination in *H. hemerocallidea* would suggest that this pathway was not the limiting factor in the germination of this seed. While the seeds imbibed, it is also possible that the applied substances were not taken up.

There is much evidence for the involvement of gibberellins and cytokinins in the breaking of seed dormancy (Jones & Stoddart 1977; Thomas 1977). Khan (1971) proposed that gibberellins and cytokinins have separate, complementary roles in the release from seed dormancy. The significant stimulation of germination in coatless seeds of *H. hemerocallidea* by GA_3 and BA applications would suggest the involvement of gibberellins and cytokinins in the release from dormancy in this species. The gibberellin enhanced germination over a wider temperature range and was associated with a more rapid initial germination than was the cytokinin, and so the endogenous gibberellin level may be more limiting. The results with the combined treatment of GA_3 and BA did not indicate an interaction of gibberellins and cytokinins in the release from dormancy in *H. hemerocallidea*. Although germination in this species was stimulated by BA, the treatments with kinetin were ineffective. Stimulation of germination by BA, though not by kinetin, has similarly been reported for other species (Biddington & Thomas 1976; Mitrakos & Diamantoglou 1984). This may be a consequence of a number of factors, including differences in the uptake of these substances, in their metabolism to an active form, or in their primary mechanism of action (Thomas 1977).

The ability of ammonium chloride to stimulate germination in some species may result from the supply of nitrogen (Hendricks & Taylorson 1974). The enhancement of germination in *H. hemerocallidea* by ammonium chloride applications would indicate that germination in this species may be partly limited by the supply of nitrogen. However, ammonium chloride applications were observed to be effective in *H. hemerocallidea* only at 25°C and 30°C, and at these temperatures were less effective than the other compounds which significantly stimulated germination. Thus, a lack of nitrogen would seem not to be a major component of seed dormancy in this species.

Thiourea applications enhanced germination in *H. hemerocallidea* seeds at all three temperatures considered, in a manner similar to GA_3. Ellis, Hong & Roberts (1983) considered that the stimulatory effect of thiourea may be related to the presence of a sulphydryl group. However, applications of 2-mercaptoethanol (which also contains a sulphydryl group) were found to be ineffective in this study. It is thus difficult to comment on the nature of dormancy in these seeds from the results of thiourea applications.

It is significant that about 60% of *H. hemerocallidea* seeds could not be stimulated to germinate by the treatments applied. This seems to be due to both a coat-imposed and embryo dormancy, the nature of which remains unknown. The deep dormancy of seeds of this species is obviously the main reason why few if any seedlings are observed in the field.

Acknowledgements

The financial assistance of Essential Sterolin Products is gratefully acknowledged.

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